

# PATENT COOPERATION TREATY

From the  
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

REC'D 19 SEP 2005

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## WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY (PCT Rule 43bis.1)

Date of mailing  
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference  
see form PCT/ISA/220

**FOR FURTHER ACTION**  
See paragraph 2 below

International application No.  
PCT/US2005/008866

International filing date (day/month/year)  
16.03.2005

Priority date (day/month/year)  
16.03.2004

International Patent Classification (IPC) or both national classification and IPC  
G01N33/50, G01N33/58

Applicant  
AMNIS CORPORATION

**1. This opinion contains indications relating to the following items:**

- ☒ Box No. I Basis of the opinion
- ☒ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

**2. FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1bis(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

**3. For further details, see notes to Form PCT/ISA/220.**

Name and mailing address of the ISA:



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Authorized Officer

Mason, W

Telephone No. +49 89 2399-2623



**WRITTEN OPINION OF THE  
INTERNATIONAL SEARCHING AUTHORITY**

International application No.  
PCT/US2005/008866

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**Box No. I Basis of the opinion**

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1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.
  - ☐ This opinion has been established on the basis of a translation from the original language into the following language, which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).
2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:
  - a. type of material:
    - ☐ a sequence listing
    - ☐ table(s) related to the sequence listing
  - b. format of material:
    - ☐ in written format
    - ☐ in computer readable form
  - c. time of filing/furnishing:
    - ☐ contained in the international application as filed.
    - ☐ filed together with the international application in computer readable form.
    - ☐ furnished subsequently to this Authority for the purposes of search.
3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
4. Additional comments:

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**Box No. II Priority**

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1. ☒ The validity of the priority claim has not been considered because the International Searching Authority does not have in its possession a copy of the earlier application whose priority has been claimed or, where required, a translation of that earlier application. This opinion has nevertheless been established on the assumption that the relevant date (Rules 43*bis*.1 and 64.1) is the claimed priority date.
2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43*bis*.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.
3. Additional observations, if necessary:

**WRITTEN OPINION OF THE  
INTERNATIONAL SEARCHING AUTHORITY**

International application No.  
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**Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or  
Industrial applicability; citations and explanations supporting such statement**

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**1. Statement**

Novelty (N)	Yes: Claims	9, 14-15, 18
	No: Claims	1-8, 10-13, 16-17
Inventive step (IS)	Yes: Claims	
	No: Claims	1-18
Industrial applicability (IA)	Yes: Claims	1-18
	No: Claims	

**2. Citations and explanations**

**see separate sheet**

**RE SECTION V**

1. The present application relates to methods for measuring movement of molecules (translocation) within (biological) cells by using fluorescent imaging. A compartment in the cell (e.g. nucleus) is identified in an image of the cell e.g by using a nuclear stain so as to form a compartment mask and in addition the area of the translocated molecules is identified from a fluorescent dye specific to such molecules. The correlation (overlap) between the region defined by the mask and the area occupied by the molecules gives a quantitative measure of the degree of cellular compartment translocation of the molecules in a particular cell - for a given cell population a histogram of correlations can be plotted to obtain a statistically meaningful measurement.

The following documents are referred to:

D1=WO0235474; D2=WO0218537.

D8="Characterization and quantitation of NF-kappaB nuclear translocation induced by interleukin-1 and tumor necrosis factor-alpha: Development and use of a high capacity fluorescence cytometric system"; Ding et al; Journal of Biological Chemistry Vol. 273, Nr. 44, pages 28897-28905; Oct. 30, 1998.

**2. CLARITY AND INTERPRETATION OF CLAIMS - ART. 6**

- "correlate". This term is sufficiently broadly worded to encompass i.a. any comparison and is not necessarily limited to a numerical (mathematical) correlation. In addition the terms should be grammatically corrected to "correlating" (claims 1, 10).
- "cell" (claims 1, 10). Should be reworded to make clear that a biological cell is intended.
- "molecular" (claims 1, 10). The presence and location of the molecules within the cell should be indicated.

- "compartment marker" (claim 1). The significance of the wording "compartment" is not evident from the wording of the claim i.e. it is not apparent what the compartment is or where it is located.
- "A cell" (claims 1, 10 final line). Should be reworded to **THE** cell to make clear that the cell previously referred to is intended.
- "creating a compartment / nuclear mask" (claims 1, 10). Wording such as "from the image of the marked cell" should be added.
- "correlating ... molecular marker" (claims 1, 10). Should be reworded to make clear that the image of the molecular marker is intended.
- "nuclear translocation". Should be reworded to "molecular translocation" since it is the movement of the molecules rather than the movement of the nucleus which is to be determined.

### 3. PRIOR ART

D1 (pages 40-41) describes a method from performing a translocation assay in which the emission of two or more fluorescently-labelled species is detected simultaneously, excited by one or more illumination wavelengths. The translocation of interest is of one or more species, which may be proteins, lipids or other molecular complexes or sub-cellular structures such as vesicles, from one well-defined region of a cell to another - examples are i.a. transcription factors (NF-KB, NFAT, AP-1). In one analysis routine to process translocation image data the labelled location is the cell nucleus, the label being a fluorophore specific for DNA, such as Hoechst 33342. The second species is a transcription factor whose migration from the cytoplasm to the nucleus is the subject of the assay. This protein can be labelled by a variety of methods, including expression as a fusion with GFP, and contacting the sample with a fluorescently-labelled antibody specific to the transcription factor protein. The routine determines the amount of a first fluorescently-labelled species that is distributed in a correlated or anticorrelated manner with respect to a second

fluorescently-labelled species. In one embodiment the analysis is used to assay the activity of a chemical compound and an XY stage scanning apparatus is used.

D2 (pages 26-28, Example 2, claims 45-51) discloses a method and a system for determining the distribution and in particular the translocation of component molecules in cell in which the molecules can be localised to a subcellular organelle, cell membrane, a cell nucleus, cell chromatin, mitochondria etc. For comparison of two components, cells are labelled with two fluorescent materials, two fluorescent images are acquired using a high-resolution CCD camera and suitable filters and the two images processed by means of an image correlation analysis. The image correlation analysis determines a global correlation coefficient, which is indicative of the level of co-localization between the two images. In principle, a correlation value of 1 indicates identity between the two images, -1 indicates inverse distribution ("positive-negative relationships") and "0" indicates no correlation. According to the principle of this method the numerical correlation is a quantitative measure of image similarity between an image from one fluorescer bound to a molecular component and localised in a subcellular compartment with another molecular component distributed in a different manner throughout the cell.

**4. NOVELTY**

In view of the interpretation of claims above and the disclosure of the above documents:

Claims 1-8, 10-13, 16-17. See D1;

Claims 1, 3-5, 10-11, 17. See D2;

- together claims 1-8, 10-13, 16-17 do not meet the requirement of novelty (Art. 33.2 PCT).

**5. INVENTIVE STEP**

The subject-matter of the remaining dependent claims does not meet the requirement of inventive step (Art. 33.3 PCT) for the following reasons:

Claims 9, 14. LPS, IL-1beta/TNF-alpha. Obvious inducing agents and molecules of interest see D8.

Claim 15. 7-AAD. Standard nuclear marker.

Claim 18. Time delay and integration. Standard technique used in fluorescent image in view of weak intensities.

6. The text in the Figures should be deleted and preferably inserted in the description referring to the corresponding Figure (R.11.11 PCT).

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**Box No. V Reasoned statement under Rule 43b/s.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

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**1. Statement**

Novelty (N)	Yes: Claims	9, 14-15, 18
	No: Claims	1-8, 10-13, 16-17
Inventive step (IS)	Yes: Claims	
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Claims 1-8, 10-13, 16-17. See D1;

Claims 1, 3-5, 10-11, 17. See D2;

- together claims 1-8, 10-13, 16-17 do not meet the requirement of novelty (Art. 33.2 PCT).

#### 5. INVENTIVE STEP

**WRITTEN OPINION OF THE  
INTERNATIONAL SEARCHING  
AUTHORITY (SEPARATE SHEET)**

International application No.

PCT/US2005/008866

The subject-matter of the remaining dependent claims does not meet the requirement of inventive step (Art. 33.3 PCT) for the following reasons:

Claims 9, 14. LPS, IL-1beta/TNF-alpha. Obvious inducing agents and molecules of interest see D8.

Claim 15. 7-AAD. Standard nuclear marker.

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